

12/18/2006  
BMH

RAN: Attorney Review of Fax sent 12/18/2006 12/16/2006

## ATTACHMENT 12

1055 Progress Circle  
Lawrenceville, GA 30043  
Phone: 678-376-1055  
Fax: 678-376-1413**Anitox Corp.****Fax**To: Rick Mayfield From: Julio L. Pimentel

Fax: \_\_\_\_\_ Date: \_\_\_\_\_

Pages: (Including cover sheet) \_\_\_\_\_

Re: \_\_\_\_\_

## 6. ANTI-NUTRITIONAL FACTORS PRESENT IN PLANT FEEDSTUFFS

Page 1 of 1



## 6. ANTI-NUTRITIONAL FACTORS PRESENT IN PLANT FEEDSTUFFS

The presence of endogenous anti-nutritional factors within plant feedstuffs is believed to be the largest single factor limiting their use within compounded animal and fish feeds at high dietary levels. Figure 1 summarizes the major groups of anti-nutritional factors present in plant foodstuffs with more specific examples given in Table 3. Although these factors vary in their individual toxicity to fish, a large proportion of them can be destroyed or inactivated by heat treatment processes (Tacon and Jackson, 1985).

Unfortunately toxicological studies have not been performed on the majority of these anti-nutritional factors; on a general basis however their presence in untreated foodstuffs normally results in anorexia, reduced growth and poor food conversion efficiency when used at high dietary concentrations (for review see NRC, 1983).



## 7. ADVENTITIOUS TOXIC FACTORS PRESENT IN FOODSTUFFS

Page 1 of 2



## 7. ADVENTITIOUS TOXIC FACTORS PRESENT IN FOODSTUFFS

Finally, nutritional pathology may arise from the presence of specific adventitious toxic factors or contaminants within certain foodstuffs, including:

### a) Intentional additives

- binders and stabilizers (carboxymethyl cellulose, alginates, gums)
- therapeutic drugs (antibiotics, sulphonamides, nitrofurans, arsenilic acid)
- growth promotants (as above, plus anabolic steroids, synthetic androgens)

### b) Toxic factors arising from processing

- solvent residues present in solvent extracted oilseeds (methylene chloride, ethylene dichloride, trichloroethylene, acetone, iso-propyl alcohol)
- lipids spoiled by oxidation and/or heat (rancidity, oxidation products).

### c) Contaminants of biological origin

- protozoan toxins from spoiled fish
- algal toxins from shellfish/fish
- fungal toxins in stored foods (i.e. aflatoxins)
- bacterial toxins from contaminated foodstuffs (i.e. botulinum toxin)
- pathogens (viable bacteria, viruses and fungi)

### d) Synthetic contaminants

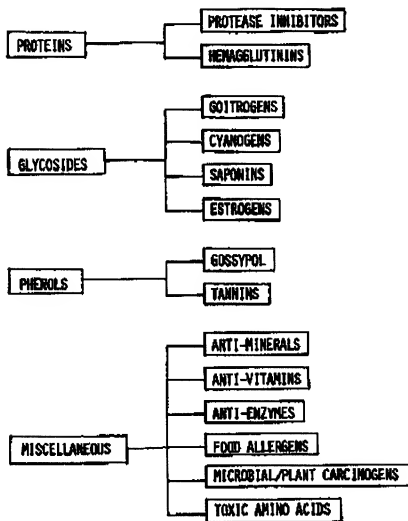
- pesticide residues (chlorinated hydrocarbons)
- organochlorine compounds (polychlorinated biphenyls)
- petroleum hydrocarbons
- heavy metals

As with plant anti-nutritional factors, dietary toxicological studies have not been performed with the majority of the above mentioned contaminants. For review see NRC (1983); Lovell (1984).

**Figure 1. Classification of endogenous toxic factors occurring in plant foodstuffs of agricultural importance according to chemical properties (adapted after Liener, 1975)**

## 7. ADVENTITIOUS TOXIC FACTORS PRESENT IN FOODSTUFFS

Page 2 of 2



**Table 3. Endogenous anti-nutritional factors present in plant foodstuffs commonly used for animal feeding**



<u>Cereals</u>	<u>Anti-nutritional factor - 1 2 3</u>		
Barley ( <i>Hordeum vulgare</i> )	x	x	-
Rice ( <i>Oryza sativa</i> )	x	x	-
Sorghum ( <i>Sorghum bicolor</i> )	x	-	-
Wheat ( <i>Triticum vulgare</i> )	x	x	-
Corn, maize ( <i>Zea mays</i> )	x	-	-
<u>Root tubers</u>			
Sweet potato ( <i>Ipomoea batata</i> )	x	-	-
Cassava, tapioca ( <i>Manihot utilisima</i> )	x	-	-
Potato ( <i>Solanum tuberosum</i> )	x	x	-
<u>Legumes</u>			
Broad, Faba bean ( <i>Vicia faba</i> )	x	x	-
Chick pea, Bengal gram ( <i>Cicer arietinum</i> )	x	-	-
Cow pea ( <i>Vigna unguiculata</i> )	x	x	-
Grass pea ( <i>Lathyrus sativus</i> )	x	-	-
Haricot, navy, kidney bean ( <i>Phaseolus vulgaris</i> )	x	x	-
Horse gram ( <i>Macrotyloma uniflorum</i> )	x	x	-
Hyacinth, field bean ( <i>Dolichus lablab</i> )	x	x	-
Lentil ( <i>Lens culinaris</i> )	x	x	-
Lima bean ( <i>Phaseolus lunatus</i> )	x	x	-
Lupin ( <i>Lupinus albus</i> )	x	-	-
Mung bean, green gram ( <i>Phaseolus aureus</i> )	x	-	-
Field pea ( <i>Pisum sativum</i> )	x	x	-
Pigeon pea, red gram ( <i>Cajanus cajan</i> )	x	x	-
Rice bean ( <i>Vigna umbellata</i> )	-	x	-
Runner bean ( <i>Phaseolus coccineus</i> )	x	x	-
Sword, jack bean ( <i>Canavalia gladiata</i> )	x	x	-
Velvet bean ( <i>Stizobolium deeringianum</i> )	x	-	-
Winged bean ( <i>Psophocarpus tetragonolobus</i> )	x	x	-
Guinea pea ( <i>Abrus precatorius</i> )	x	x	-
Carob bean ( <i>Ceratonia siliqua</i> )	x	-	-
Guar bean ( <i>Cyamopsis tetragonoloba</i> )	x	-	-
Alfalfa, lucerne ( <i>Medicago sativa</i> )	x	-	-
Black gram ( <i>Phaseolus mungo</i> )	x	-	-
Ipil ipil ( <i>Leucaena leucocephala</i> )	-	-	-
<u>Oilseeds</u>			
Groundnut ( <i>Arachis hypogaea</i> )	x	x	-
Rapeseed ( <i>Brassica campestris napus</i> )	x	-	x
Indian mustard ( <i>Brassica juncea</i> )	x	-	x
Soybean ( <i>Glycine max</i> )	x	x	x
Sunflower ( <i>Helianthus annuus</i> )	x	-	-
Cottonseed ( <i>Gossypium</i> spp.)	-	-	-
Linseed ( <i>Linum usitatissimum</i> )	-	-	-
Sesame ( <i>Sesamum indicum</i> )	-	-	-
Cumbe, Abruscinus cathartica ( <i>Abrus cathartica</i> )	-	-	-

---

## Anti-nutritional Factors in Oil Seeds as Aflatoxin in Ground Nut

By Mrs. Farzana Panhwar, February 2005

---

**Author:** Farzana Panhwar (Mrs)  
**Address:** 157-C, Unit No.2, Latifabad, Hyderabad (Sindh), Pakistan  
**E-mail:** farzanapanhwar@hotmail.com  
farzanapanhwar@yahoo.com  
**Fax:** 92-21-5830826 and 82-221-860410  
**Publisher:** Digitalverlag GmbH, Germany  
www.ChemLin.com

© 2005 Digitalverlag GmbH, Germany

Editor ChemLin

www.ChemLin.com

**ANTI-NUTRITIONAL FACTORS IN OIL SEEDS AS AFLATOXIN IN GROUND NUT.****Abstract.**

- Brazil nut:- It contain sufficient amount of barium to cause poisoning. The kernel of brazilian nut on the air-dry basis contains 0.26% barium. Extraction of the nut - meat with water acidified with hydrochloric acid removes all the barium.
- Some times physiological changes also cause the formation of poisonous substances in oil seeds.
- Rhubarb and spinach contain oxalic acid, which bind with calcium so it creates calcium deficiency.
- The oil-seeds contain anti-nutritional and toxic factors that must be inactivated if their full value to be realised.
- Oxalate found in Sesame oil. Oxalic acid forms insoluble salts with calcium, magnesium and iron, and act to prevent these minerals from being absorbed during digestion.
- Phytic acid present in cereals:- It is scientifically is Inositol hexaphosphonic acid. It is a phosphoric acid ester of magnesium salt is called phytin. Phytic acid forms insoluble salts with calcium, iron and magnesium, it interferes with the intestinal absorption of these minerals. Phytic acid is associated with fibre.
- Phytes also results into decrease iron and zinc absorption. Cereals contain Phytin, its bind with calcium so cause calcium deficiency.

Cotton seeds endosperm has pigment glands which contain the toxic pigment gossypol. The gossypol that gets into the oil is removed during oil refining. Gossypol that stay with the meal has problem accepting cotton seed flour and cotton seed protein for human food. It is possible to remove un-ruptured pigment glands by controlled disintegration of the seed in the hexane and centrifugal separation of lighter glands form the rest of endosperm. Glandless varieties of cotton seeds are free of gossypol have been developed by plant breeder.

Some molds produce poisonous called mycotoxin. The mycotoxin aflatoxin found in ground nut. If eaten, it can cause severe and some times deadly liver damage. Aflatoxin-producing molds grow under warm, moist conditions, care is needed to store these food.

---

Anti-nutritional Factors in Oil Seeds as Aflatoxin in Ground Nut**Anti-nutritional Factors in Oil Seegs as Aflatoxin in Ground Nut****Introduction.**

Anti-nutritional factors are those substances found in most foods, and they are poisonous or in some way limit the nutrients available to the body. Plants have evolved these substances to protect them-selves from being eaten. Since anti-nutrient occur in small quantities that they cause no harm. But if the diet is not varied some of these toxins built up in the body to harmful levels. The same way traditional food processing techniques are used to eliminate any harmful effects of anti-nutrients occurs in the diet.

**Some oil seeds and their botanical names are as under:**

Oil Seeds	Botanical names
• Black mustard.	Brassica nigra Koch.
• Brown Sarson.	Brassica campestris var. dichotoma.
• Castor.	Ricinus communis L.
• Coconut.	Cocos nucifera L.
• Ground nut.	Arachis hypogaea L.
• Rape seed.	Brassica campestris L.
• Linseed.	Linum usitatissimum L.
• Niger.	Guizotia abyssinica cass.
• Safflower.	Carthamus tinctorius L.
• Sesamum.	Sesamum indicum L and Sesamum Orientale L.
• Soybean.	Glycine max Merr.
• Sunflower.	Helianthus annuus L.

---

Anti-nutritional Factors in Oil Seeds as Aflatoxin in Ground Nut

---

**Anti-nutritional/toxic constituents in oil-seed.**

<b>Oil Seed.</b>	<b>Anti-nutritional/Toxic Constituents.</b>
• Ground nut, Rape seed and Mustard.	Aflatoxin, goitrogenic factors (red skin). Glucosinolates.
• Seame.	Oxalates (husk), phytes.
• Soybean.	Trypsin inhibitors, haemagglutinins, goitrogenic factors.
• Safflower.	Bitter and cathartic factors.
• Cotton seeds.	Gossypol.

**Anti-nutrients found in ground nuts and other oil seed and their effects.**

<b>Anti nutrient</b>	<b>Effect on body</b>
• Phytes.	Reduce Ca and Fe absorption.
• Oxalates.	Reduce Ca absorption, encourage kidney stone formation.
• Oligosaccharides.	Produce intestinal gas, discomfort, loss of appetite.
• Hydrogen Cyanid (HCN).	Contribute to goiter if it is low in iodine.
• Aflatoxin.	It cause liver damage and cancer.

Some vitamins in food may be destroyed by anti-nutritional substances. Ascorbase is an enzyme released by plant cells in response to damage such as harvesting. This enzyme start a reaction changing ascorbic acid to oxalic acid, an oxalate, this reaction increase during storage, especially in warm climate. It is best to harvest close to the time of eaten.

---

Anti-nutritional Factors in Oil Seeds as Aflatoxin in Ground Nut

**Groundnut or peanut as an example.**

- In the year 1994-95 the area under ground nut production in Pakistan was 96,600 hectares while in Sindh was 3,600 hectares. Ground nut production in Pakistan was 105,700 tonnes while in Sindh its production was 6,800 tonnes. For the same year the yield per hectare in Pakistan was 1,094 kgs while in Sindh the yield per hectare in Sindh was 1,888 kgs.
- Groundnut flour show protein efficiency ratio at 10% level is 1.65.
- Total tocopherol 0.93mg/g of oil, in which alpha-tocopherol is 36.4% of the total and gamma tocopherol is 64.1% of the total while delta tocopherol is absent in ground nut.
- Loose shell kernels before storage, using a market standard of 7% moisture content this removal of foreign material and loosed-shelled kernel will significantly reduce excess moisture and initial fungal growth pockets.
- In peanut oil content, iodine value, tocopherol, total and protein nitrogen and total sugar content are not influenced by storage moisture. Carbonyl compound free fatty acids and peroxide values varied directly with storage moisture.

**Aflatoxin.**

- Aflatoxin is the term applied to the toxic metabolites produced by some strains of fungus *Aspergillus flavus* a pathogens which may grow on numerous commodities in various parts of the world, including peanut. Aflatoxin-contaminated peanut are inedible, their use is limited to the production of pea nut oil.
- Aflatoxin a poisonous toxin which is produced by the fungus *Aspergillus flavus* found in ground nut meal. However fungus is also found on cotton seeds, palm-kernel even in maize. The toxin causes a reduction in milk yield and growth rate in cattle if they consume groundnut meal.
- Although more than dozen aflatoxin have been identified but the important are; Aflatoxin B<sub>1</sub>, M<sub>1</sub>, G<sub>1</sub>, B<sub>2</sub> and G<sub>2</sub>. Aflatoxin B<sub>1</sub>, G<sub>1</sub>, B<sub>2</sub> and G<sub>2</sub> are synthesized in large quantities chemically aflatoxins are substituted coumarins that are small molecules. Aflatoxin are not protein as are many of toxins produced by bacteria. B<sub>1</sub> is the most toxic carcinogenic.
- The aflatoxin are heat-stable but can be degraded by strong acid or alkaline solution, oxidising agent, bisulfite can degrade aflatoxin.
- Aflatoxins also present in wheat, spaghetti, corn, rice, sorghum, oat, Rye, barley and other cereals.

---

**Anti-nutritional Factors in Oil Seeds as Aflatoxin in Ground Nut**

---

Water stress during maturity of groundnut combined with insect damage helps *Aspergillus flavus* in inciting infection leading to aflatoxin production in the field. This fungus is also present in pulses, groundnut and oilseeds, optimum conditions for aflatoxin production are 25-30°C with 85% relative humidity in the atmosphere. Fungus only grows when the moisture content of seeds exceeds 9%. It mostly happened due to poor storage conditions which allow pods or kernels to become damp and contaminated by fungus.

Some molds produce poisonous called mycotoxin. The mycotoxin aflatoxin found in ground nut. If eaten, it can cause severe and some times deadly liver damage. Aflatoxin-producing molds grow under warm, moist conditions, care is needed to store these food.

**Aflatoxin can be controlled by following ways.**

- Harvesting the produce at the right maturity when lower leaves die and fall, the shell hardens, the pods begin to darken internally and seed coat (testa) darkens.
- The produce should be lifted from soil with minimum damage.
- Pods should be dried quickly so as to contain less than 8% moisture.
- Produce should be stored in well ventilated store houses in unshelled condition.
- Insecticides should be applied to avoid damage by storage pest.
- Contaminated seeds should be removed.

**Conclusion.**

- Peanuts, having moisture at the time of harvest, support the growth and development of aflatoxins. In order to avoid this situation the peanuts are stored under conditions to control molds growth and carefully inspected to minimise this hazard. Aflatoxin can be removed from peanut meal by solvent extraction, and inactivated by oxidising agents and other treatments.
- Anti-nutritional factors are studied by nutritionists and toxicologists. The new analytical tools of gas and liquid chromatography as well as different types of spectroscopy, through knowledge of the composition of oil-seeds as well as chemical nature of minor constituents are studied.

---

Anti-nutritional Factors in Oil Seeds as Aflatoxin in Ground Nut

---

**Future strategies in oil seeds development are as under:**

- Expansion of area on agro-ecological conditions.
- Conservation of germplasms-plant genetic resources must preserve superior germplasms.
- Production of quality seeds-we make available certified seeds of improve varieties.
- Biotechnology-strengthen tissue culture techniques.
- Rhizobial cultures - supply rhizobial culture for ground nut.
- Post-harvest technology-improved processing, storage and extraction methods.

**References.**

- 1) Frederic Rosengarten, Jr., the book of edible nut; Walker and Co., New York, 1984, 384 p.
- 2) Norman N. Potter; Food Science; Third edition; CBS Publishers and Distributors; 1987; 780 p.
- 3) Norman W. Desrosier, James N. Desrosier, The technology of food preservation; Fourth edition; AVI Publishing Co. Inc., 1978, 558 p.
- 4) George F. Stewart and Maynard A. Amerine; Introduction of food Science and Technology; Academic Press, New York; 1973; 294 p.
- 5) Howard O. Trlbold; Leonard W. Aurand; Food composition and analysis; D. Van Nostrand Co., Inc., 1982; 496 p.
- 6) Howard O. Trlbold; Leonard W. Aurand; Food composition and analysis; D. Van Nostrand Co., Inc., 1982; 496 p.
- 7) Harold, E. Pattee; Evaluation of quality of fruits and vegetables; AVI Publishing, Inc., 1985; 410 p.
- 8) Government of Pakistan Ministry of Food, Agriculture and Livestock, Economic Wing, Islamabad; Agricultural Statistics of Pakistan, 1994-95; 290 p.
- 9) Satyabrata Maiti, M.R., Hedge and S.B., Chattopadhyay; Handbook of annual oilseed crop; Oxford and IBH Publishing Co., Pvt. Ltd; 1988; 325 p.
- 10) Mahabal Ram; High yielding varieties of crops; Second edition; Oxford and IBH Publishing Co., 1986; 708 p.

---

Anti-nutritional Factors in Oil Seeds as Aflatoxin in Ground Nut

- 11) Bringi, N.V; Non traditional oilseeds and oils of India; Oxford and IBH Publishing Co. Pvt. Ltd.; 1987; 254 p.
- 12) International Seminar on Dryland Agriculture in Pakistan; November 6-8, 1988, Lahore Pakistan, Fauji Fertilizer Co., Ltd, 301 p.
- 13) David A. Cleveland and Daniela Soler; Food from dry-land gardens; An ecological nutritional, and social approach to small-scale house hold food production by Centre for People, Food and Environment USA, 1991; 387 p.
- 14) Gerald Reed; Prescott and Dunn's Industrial Microbiology; 4<sup>th</sup> Edition; CBS Publishers and Distributors; 1987; 883 p.

---

Author: Farzana Panhwar (Mrs)  
Address: 157-C, Unit No.2, Latifabad, Hyderabad  
(Sindh), Pakistan.  
E-mail: farzanapanhwar@hotmail.com  
farzanapanhwar@yahoo.com  
Fax: 92-21-5830826 and 92-221-860410  
Publisher: ChemLin  
http://www.chemlin.com  
Feb 22, 2005

---

More articles of Mrs. Farzana Panhwar see: <http://www.ChemLin.de/publications/index.htm>

To publish your research papers please contact [office@ChemLin.de](mailto:office@ChemLin.de)

## Anti-nutritional Factors in Food and Animal Feeds

Anti-nutritional factors can cause detrimental effects on humans and animal growth and performance by impairing intake, uptake or utilisation of other food and feed components, or by causing discomfort and stress to humans and animals. These anti-nutritional factors mainly occur in pulses and grain legumes and foods and feed material prepared from grain legumes and pulses.

The Food and Agricultural Chemistry Section of the Chemistry Centre can analyse a large range of anti-nutritional factors in pulses and grain legumes and food and feeds derived from these materials. These analyses complement the extensive range of nutritional analyses (i.e. protein, mineral, dietary fibre and amino acid) also carried out by the group.

### Analyses:

- Glycoalkaloids
- Phytate (myoinositol or isositol hexaphosphate)
- Tannins (total and condensed)
- Aflatoxins
- Patulin
- Oligosaccharides
- Peanut Allergen
- Trypsin and Chymotrypsin Inhibitor activity
- Vicine/Convicine
- Alkaloids (Lupin)
- Lectins (Haemagglutinins)
- Agel Toxins
- Nitrates and Nitrites

### For further information:

Contact: David Harris  
Phone: (08) 9222 3036  
Fax: (08) 9325 7767  
Email: dharris@ccwa.wa.gov.au

or

Contact: Ken Dods  
Phone: (08) 9222 3461  
Fax: (08) 9325 7767  
Email: kdods@ccwa.wa.gov.au

Website: [www.ccwa.wa.gov.au](http://www.ccwa.wa.gov.au)  
Address: 125 Hay Street, East Perth, WA 6004



The Chemistry Centre (08) 9222 3036 is a member of Chemlink Global for quality assurance (ISO 9001:2000) and has a Quality Management System certified to ISO 9001:2000 (No. 015016712).